
Biotelemetry Implant Volume and Weight in Rats: A Pilot Study Report

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Summary

This paper reports the results of a pilot study in which a 240-gram rat was implanted for 41 days with biotelemetry devices weighing a total of 36 gm (18 cc). The implanted animal showed no differences in weight gain, food and water consumption, and postnecropsy organ weights when compared to both an unoperated control animal and an animal that underwent surgery but did not receive an implant. The implanted animal also had temperature and activity rhythms similar to those reported using much smaller implants. Thus, this pilot study showed that a 240-gm rat could be implanted with biotelemetry devices weighing nearly 15% of body weight without significant changes in health or behavior. A larger study involving more animals and similar implant sizes is recommended.

Introduction

An Animal Biotelemetry System (ABS) is being planned for acquiring physiological measurands from experimental rats housed on the Space Station. The ABS is being designed to meet the science requirements stated in the Centrifuge Facility Level II Science Requirements Document (1989) and the engineering requirements in the Centrifuge Facility Flight System Specifications (1992). To meet these ambitious requirements, implanted telemetry devices will have to be larger than those available on the market today. It is necessary, therefore, to determine if a larger package can be implanted in a rat without adversely affecting the animal's health and behavior.

Telemetry devices used for intraperitoneal (IP) and subcutaneous implantation in rats today are typically 3–6 cc in volume and weigh 6–10 gm. These devices provide 1–4 channels of multiplexed data per animal. Data sampling rates are on the order of 100–300 samples per second (sps)/channel, and transmitter on/off is manually controlled via a magnetic wand or radio-frequency (rf) switch. The ABS is currently being required to acquire up to 8 rf channels per animal, sample at total rates near 20K sps, remotely control on/off, and provide animal and data identification. Preliminary engineering analyses suggest that using

current technology an implant ~15 cc in volume and 30 gm in weight could support, at a reasonable cost, most of these requirements.

To determine if rats can accommodate this size implant without health or behavioral problems, we have proposed to implant 250-gm rats with off-the-shelf telemetry transmitters and “dummy” implants (see appendix A, NASA ACUC Protocol #92-037A). In preparation for this study, we conducted a pilot study in which a single 240-gm rat was implanted with an rf transmitter (IP) and three dummy implants (subcutaneous) weighing a total of 36 gm (18 cc). This increased the animal's body weight 15%. The weight increase is less than that of a pregnant rat, which can be as much as 85 gm (Baker, 1979). It is approximately the percent body weight increase realized with the 4-gm telemetry implants used in large mice. Implantation of small osmotic pumps of similar volumes (2×6.5 -cc pumps) has been accomplished in rats for 2–4 weeks without noticeable effects (e.g., Fujinaga and Mazze, 1986). The goal of this pilot study was to verify that a rat can accommodate a large implant without significant health or behavioral changes, and to test surgical techniques, procedures, and biotelemetry equipment in preparation for a larger study using up to 25 animals. The results of the pilot study are reported here.

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Materials and Methods

Animals

Three male Sprague–Dawley rats were obtained (Simonsen Laboratories, CA) at 48 days of age and were housed individually in standard animal cages (D17 in. $\times$ W8 in. $\times$ H8 in.). They were placed in an isolated animal room at 72°F with a light:dark cycle of

12L:12D. Animals were fed standard Purina rat chow pellets and watered *ad libitum*. The care and treatment of animals conformed with the guidelines set forth in the Ames Research Center Animal Users Guide (AHB 7180-1).

Data Acquisition Equipment

One 6-gm (3-cc) temperature/activity biotelemetry implant (model #TA10TA-F40) and three 10-gm (5-cc) dummy implants (F50 packages) were obtained from Data Sciences, Inc. (DSI). Both types of packages were encapsulated in a biocompatible material and had rectangular cross sections ($\sim 9 \times 14$ mm) and rounded edges. Two RLA1000 receivers, a BCM Consolidation Matrix, and a 110-volt power supply were also obtained from DSI. The body temperature and activity data were recorded by DSI-provided software, Dataquest IV 2.0, with an Everex Step 486/33 personal computer.

Surgeries

Animals were 54 days old and approximately 240 gm in weight at the time of surgery. The Ames Animal Care Facility (ACF) veterinarian was on hand to observe and advise during the surgical procedures. One of the three rats was randomly selected for implantation and was injected intraperitoneally with 0.15 ml of Telazol (50 mg/ml). An additional dose of 0.075 ml was required

to fully anesthetize the animal. The ventral and dorsal sides of the rat were shaved and cleaned with an alcohol wipe. The skin over the lower portion of the abdomen was incised and the underlying muscle gently teased away to allow access to the peritoneal cavity. The temperature/activity transmitter was tucked into the cavity, the overlying musculature closed with 5.0 silk, and the incision closed with an animal skin clip. However, this animal did not remain fully anesthetized, even with additional doses of Telazol, and the remaining units were not implanted. The transmitter was removed and this animal became a nonimplanted-operated control. After the transmitter was removed, the animal did become fully anesthetized and remained anesthetized for approximately 6 hours.

A second rat was then selected and injected with 0.22 ml (IP) of an anesthetic cocktail mix of Ketamine, Zylazine, and Acepromazine (table 1). This animal was quickly and fully anesthetized. The transmitter was implanted intraperitoneally as described above. The animal was then placed on its stomach and an incision was made across its dorsal side. Three pockets were made beneath the skin, one rostrally and two caudally on either side of the spine (fig. 1). Each of the three dummy units was slipped snugly into one of the pockets, and the incision closed with animal skin clips. Implants were not sutured to underlying tissue. This animal remained anesthetized for approximately 1 hour. The third rat was an unoperated control.

Table 1. Anesthetic cocktail^a

Drug	Concentration, mg/ml	Volume, ml	Dose, mg/kg
Ketamine HCL (Ketaset; Aveco)	100	20	55
Zylazine (AnaSed; Lloyd Laboratories)	20	10	5.4
Acepromazine Maleate (PromAce; Aveco)	10	3	0.81

^aRecommended dosage is 0.9 ml/kg body weight, IP. Deep anesthesia typically lasts less than 1 hour in normal adult male rats. Boosters are given at 20–25% of the original dose. Anesthetized animals should be kept isothermic.

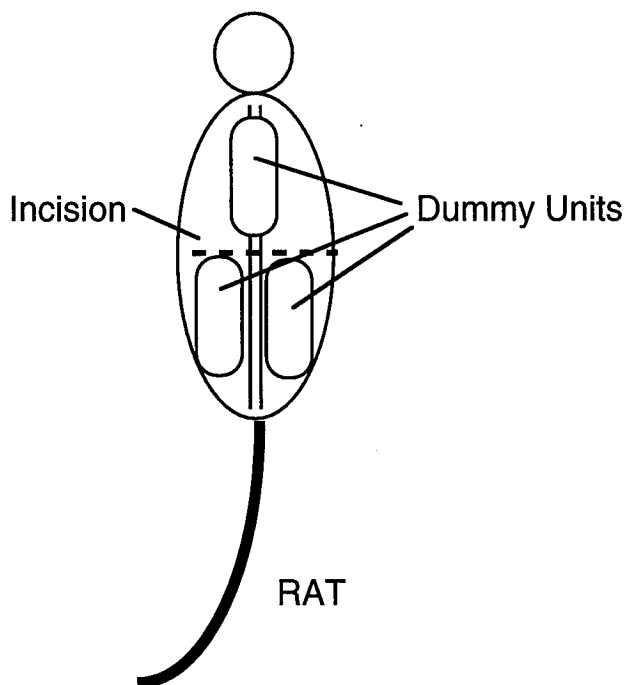


Figure 1. Location of implants. View is from above. Three 5-cc dummy devices were implanted subcutaneously on the dorsal side of the animal as shown. The actual biotelemetry unit was implanted intraperitoneally from the ventral side (not shown).

Food, Water, and Animal Weight Measurements

All three rats were weighed over a 6-day period prior to surgeries and on the day of surgery. Food and water consumption were measured each day for 3 days prior to surgery and the day of surgery. Following surgeries, these measurements were made each day for the next 10 days and every 2–3 days for the following 31 days. Animals were usually weighed, fed, and watered between 2 and 3 p.m. each day. Statistical comparisons between treatment groups were not done because there was only one animal in each group.

Temperature and Animal Activity

Temperature and animal activity were measured continuously via rf telemetry from the implanted rat using the Dataquest IV software. Temperature was sampled at 10 Hz and averaged over a 10-second period. These temperature data points were acquired every 5 minutes. Animal activity data points were obtained by summing the number of times the rf signal strength at the receiver varied beyond a predetermined threshold over a 5-minute period. In the present study this threshold was factory-set by DSI. Two receivers were used, one early in the study and one later, in order to compare their performances.

Because animal maintenance activities (cage cleaning and animal room cleaning), the measurement of food and water consumption, and animal weighing were likely to influence temperature and activity data, the animal room was sealed off for selected days during the 41-day postsurgery period. This provided relatively undisturbed recordings during these days.

Euthanasia and Tissue Samples

All three rats were decapitated 41 days following surgery. The ACF veterinarian was on site to examine the implant areas and local tissue for necrosis, local edema, and tissue inflammation. The thymus, heart, liver, adrenals, testes, and kidneys were removed from each animal and immediately weighed. The left and right gastrocnemius, soleus, and plantaris muscles from the rear limbs were also dissected and weighed. Again, statistical comparisons between treatment groups were not done because there was only one animal in each group.

Results

General

The IP implantation of the 6-gm (3-cc) temperature/activity transmitter is a standard procedure, and was carried out without incident. The implantation of three 10-gm (5-cc) dummy units subcutaneously on the backside of the animal was also surgically uneventful. Both operated animals did well following surgery. The nonimplanted-operated rat was the slowest to recover, probably due to the additional doses of anesthetic, but by the second day was doing well. The implanted animal exhibited difficulties with locomotion and coordination upon waking from anesthesia, but appeared to adapt quickly to its new load. All animals were very active by 24 hours postsurgery and appeared to be moving, grooming, eating, and drinking normally.

The subcutaneous unit implanted between the shoulder blades rotated ~90 deg soon after surgery and then slowly migrated over the left shoulder and down along the animal's left side. It stabilized by ~2 weeks postsurgery and remained along the animal's left side for the rest of the test period. The two units implanted on either side of the spinal column, caudal to the first implant, were fairly stable. However, over the duration of the test, the units slowly shifted to locations more along the sides of the animal where they eventually stabilized. The implants did not appear to cause any behavioral or health problems for the animal when located in these positions.

Food and Water Consumption

Both before and after surgery, food consumption levels were between 20 and 30 gm/day for each of the three animals (fig. 2). However, immediately following surgeries food consumption fell dramatically to ~10 gm/day for both the implanted animal and the nonimplanted-operated animal. In both cases values recovered to near presurgical levels by the second day postsurgery. As expected, food consumption remained unchanged in the control animal. For all three animals, food consumption began to decline toward the end of the test period as the animals matured.

Water consumption for the implanted and the non-implanted-operated animal was similar (fig. 3). Before and after surgery, both animals showed water consumption levels between 25 and 35 ml/day. Interestingly, the control animal showed water consumption levels 15%–30% higher than the operated animals. This higher level of consumption was apparent both before and after the surgeries, though the difference increased slightly toward the end of the test period (as much as 50% greater than the operated animals). None of the three animals

showed any clear drop in water consumption immediately following surgery.

Weight Gain

All three animals showed similar growth patterns and nearly identical growth rates over the 48-day test period (fig. 4). Animal growth rates were 8–9 gm/day at the beginning of the study (animal weights near 200 gm) and decreased steadily to 2–3 gm/day at the end of the test period (animal weights near 390 gm). The only exception was during the 2–3 day period following surgery. During this period the control animal showed a fairly steady weight increase of 2–3% per day (6–7 gm/day). The implanted animal showed an initial decrease in weight of 4% the first day after surgery, and weight increased at a rate just under that of the control animal thereafter. The nonimplanted-operated rat exhibited a somewhat larger weight decrease (5–6%) the first day following surgery, and a smaller decrease continued through the second day. By the third day postsurgery, growth rates in the non-implanted-operated animal had returned to normal and were similar to those of the control animal.

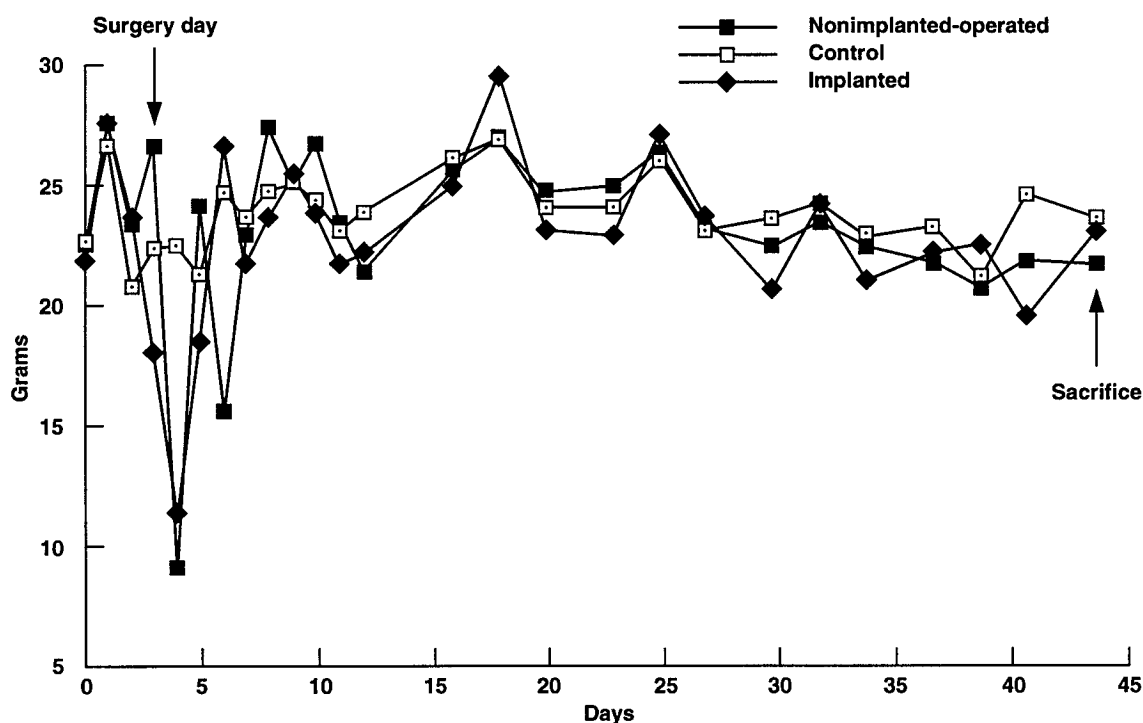


Figure 2. Daily food consumption in grams for 3 days prior to surgery, the day of surgery, and 41 days following surgery for implanted, control, and nonimplanted-operated rats. Plotted values are the weight of food consumed per day for the time between the afternoon of the day indicated and the afternoon of the previous day.

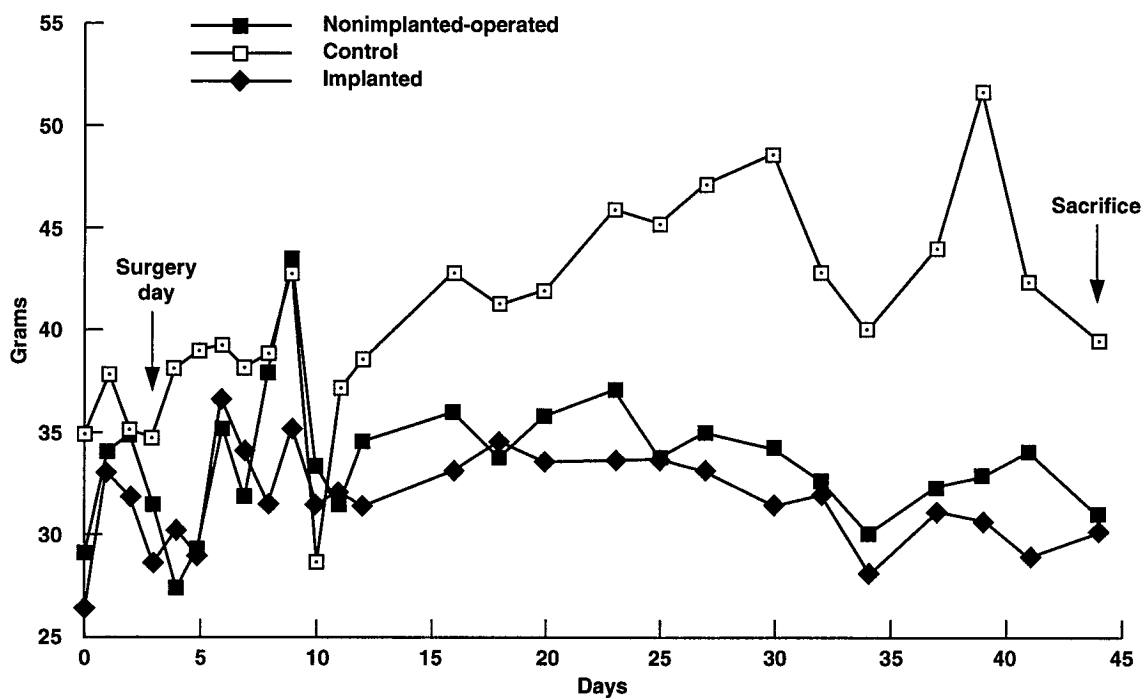


Figure 3. Daily water consumption in grams for 3 days prior to surgery, the day of surgery, and 41 days following surgery for implanted, control, and nonimplanted-operated rats. Plotted values are the weight of water consumed per day between the afternoon of the day indicated and the afternoon of the previous day.

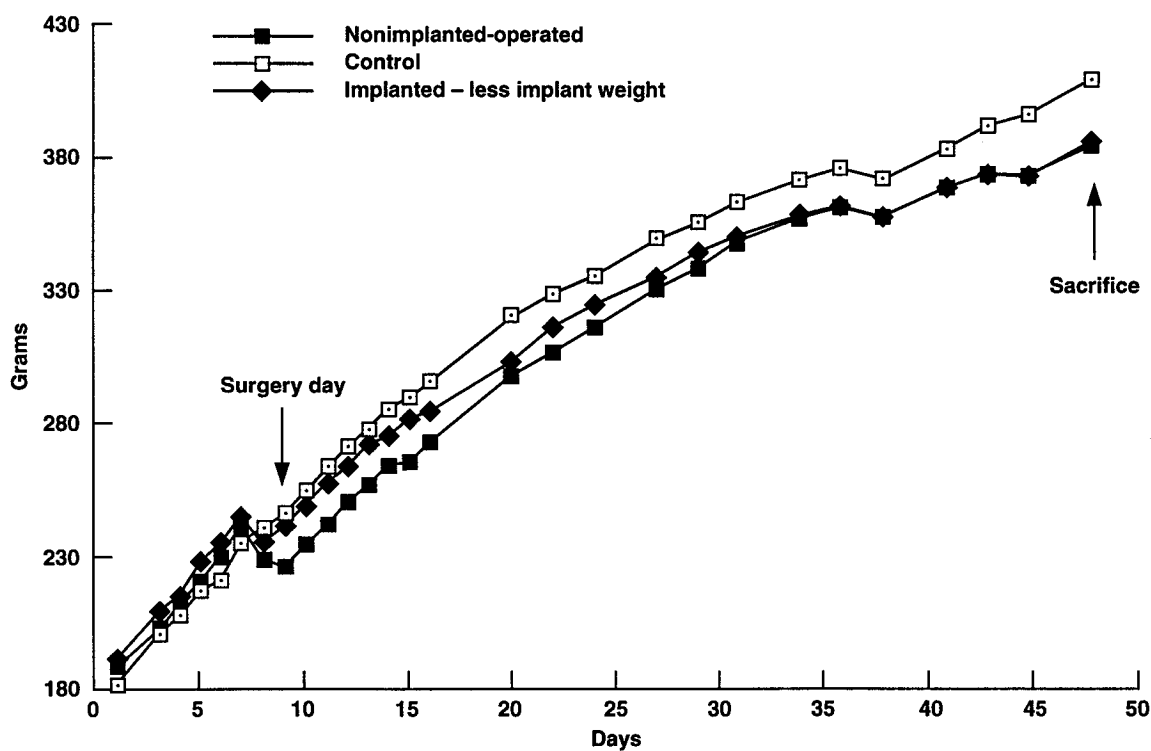


Figure 4. Animal weights over a 6-day period prior to surgery, the day of surgery, and 41 days following surgery for implanted, control, and nonimplanted-operated rats. The weight of the implanted animal is corrected for the weight of the implants.

RF Data

The biotelemetry equipment obtained from DSI performed well during this study. Calibrated temperature values were in a nominal range (36°–38°C) and showed the expected diurnal responses. The receivers provided good coverage across the 8 × 17-inch floor space and 8-inch cage height. Dropouts were less than 1%. Cross-talk between receivers was eliminated by separating adjacent receivers by 20 inches or more.

Temperature— Examples of selected temperature data are shown in figure 5. Figure 5(a) shows a 24-hour temperature profile starting at 4:45 p.m., 6 days post-surgery, approximately 30 minutes prior to the start of the 12-hour dark cycle. During the 12-hour dark cycle, body temperature values ranged between 37° and 38°C, averaging near 37.5°C. Body temperature dropped during the following 12-hour light cycle to values ranging between 36.5° and 37.5°C, averaging near 37°C. Very few dropouts were observed; only 1% in this particular time period. Figure 5(b) shows similar temperature cycles over a 4-day period starting 23 days postimplantation. Again, body temperature varies between approximately 37.5°C at night and 37°C during the day with peak values near 38°C and minimum values near 36.5°C. Dropouts were less than 1%. Figure 5(c) shows temperature cycles over a 7-day period starting 34 days after implantation. Temperature variations and dropouts are very similar to those seen during earlier periods, as shown in figures 5(a) and 5(b).

Activity— Examples of selected activity data are shown in figure 6. Data are shown for the same times in which temperature data are presented. Figure 6(a) shows activity measures over a 24-hour period starting 6 days post-surgery. The activity level of the animal was clearly greater during the dark period than during the lights-on period. During the 12-hour dark period the animal

averaged 25 counts per 5-minute interval with values ranging from 0 to 130. During the following light period, activity decreased to an average of 9 counts per 5-minute interval and values ranged between 0 and 140. Figure 6(b) shows diurnal variations in activity level over a 4-day period starting 23 days postsurgery. For example, during the dark period of the first cycle, the average number of counts per 5-minute interval was 17 and during the lights-on period was 5. Figure 6(c) shows diurnal activity cycle over a 7-day period starting 34 days after surgery. On the first cycle, during the dark period, the number of counts per 5-minute interval was 16, and during the lights-on period was 6. Figures 6(b) and 6(c) show data acquired using a different receiver from that used to acquire the data shown in figure 6(a).

Postmortem Necropsy and Organ Weights

Following euthanasia the transmitter and the three dummy implants were removed from the implanted animal. All implants were easily removed; no tissue adhesion was apparent. The ACF veterinarian was on hand for a visual necropsy, and found no obvious tissue abnormalities. There appeared to be some thickening of the mesentery, but there were no signs of tissue infection, edema, or similar abnormality in the areas of the IP or subcutaneous implants. The tissues looked virtually indistinguishable from the control animal.

Table 2 shows the organ wet weights from each of the three animals. Both raw weights and weights normalized to 100 gm of animal weight at sacrifice are tabulated. There were no differences between animals greater than 26% for any of the organ weights. A larger statistical sample would be required to evaluate the differences observed in this study.

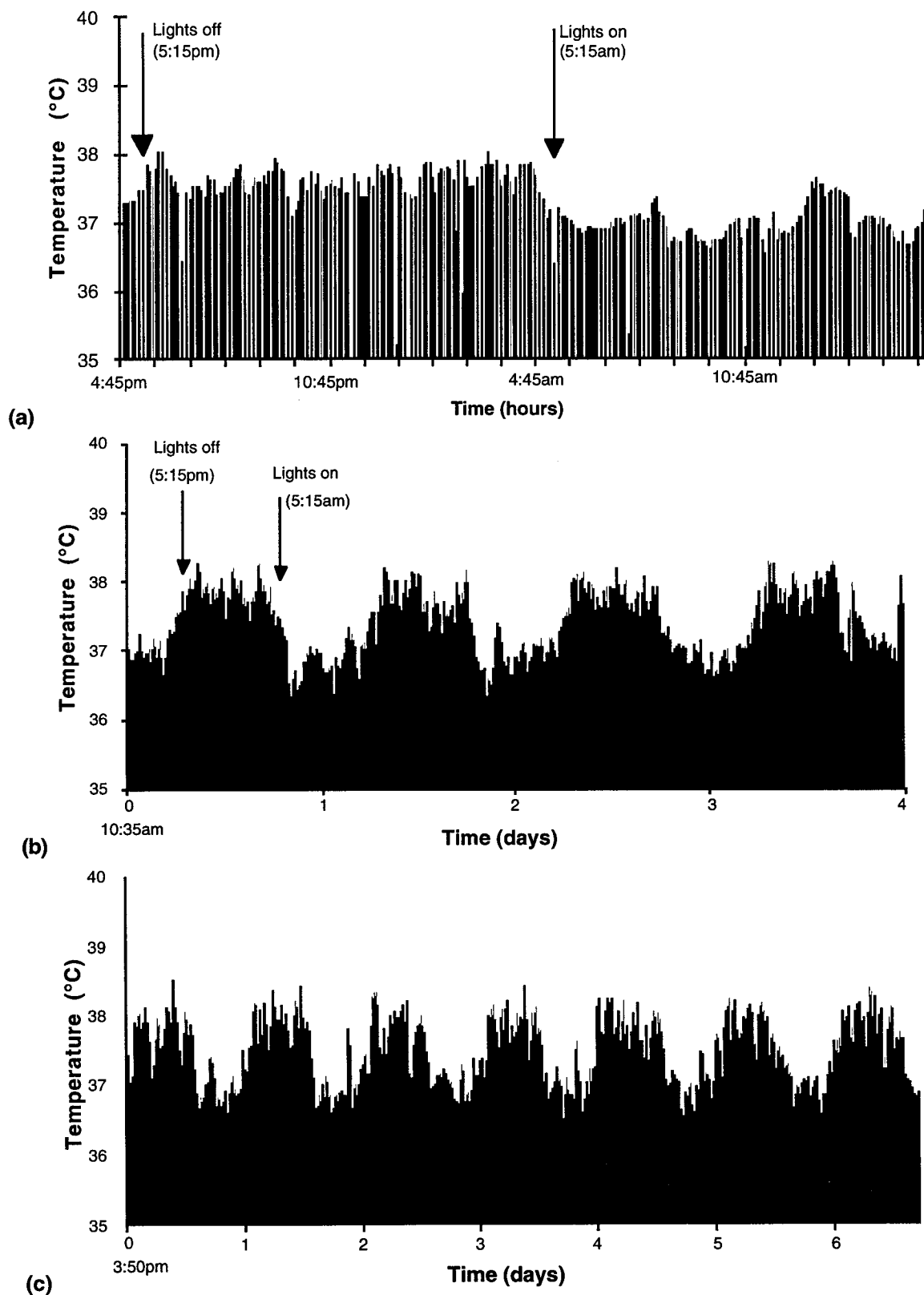


Figure 5. Temperature changes for the implanted rat over (a) an ~24-hour period starting 6 days after surgery, (b) a 4-day period starting 23 days after surgery, and (c) a 7-day period starting 34 days after surgery. Lights were turned off at 5:15 p.m. each day and turned on at 5:15 the following morning.

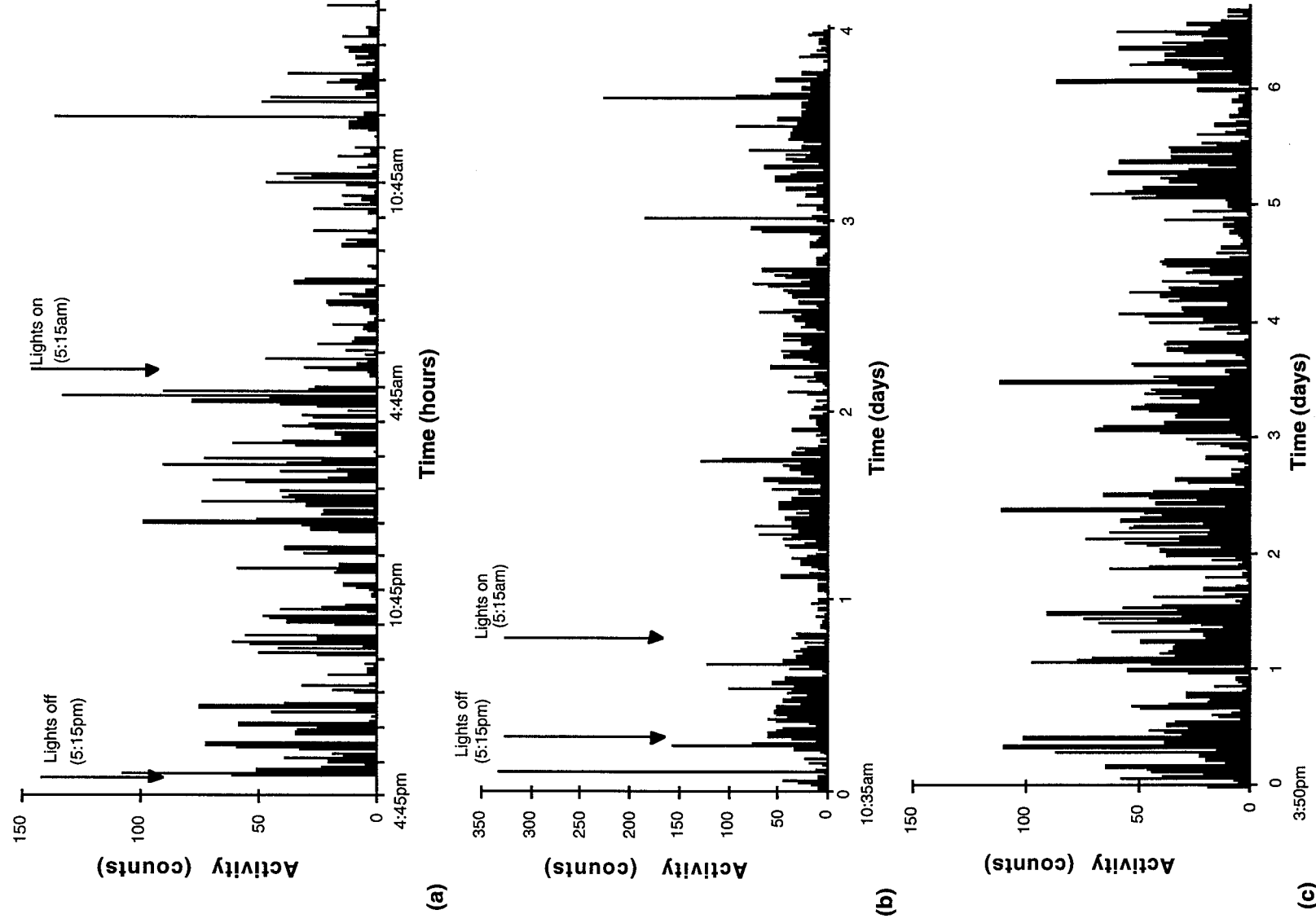


Figure 6. Activity changes in the implanted rat over (a) an ~24-hour period starting 6 days after surgery, (b) a 4-day period starting 23 days after surgery, and (c) a 7-day period starting 34 days after surgery. Lights were turned off at 5:15 p.m. each day and turned on at 5:15 the following morning.

Table 2. Wet weights of dissected tissues^a

Organ weight						
	Adrenals (R + L) mg	Thymus mg	Heart gm	Testes (R + L) gm	Kidney (R + L) gm	Liver gm
Rat 1	53	444	1.228	3.051	2.387	13.712
Rat 2	58	515	1.349	3.268	2.754	15.675
Rat 3	46	443	1.211	3.399	2.492	13.526
Muscle weight						
	Gastroc (R) gm	Gastroc (L) gm	Soleus (R) mg	Soleus (L) mg	Plantaris (R) mg	Plantaris (L) mg
Rat 1	2.317	2.339	160	150	442	444
Rat 2	2.225	2.350	153	135	452	418
Rat 3	2.199	2.153	146	147	401	387
Organ weight per 100 gm body weight						
	Adrenals (R + L) mg	Thymus mg	Heart gm	Testes (R + L) gm	Kidney (R + L) gm	Liver gm
Rat 1	13.8	115.6	0.320	0.794	0.621	3.570
Rat 2	14.2	126.0	0.330	0.799	0.674	3.834
Rat 3	11.9	114.6	0.313	0.879	0.645	3.500
Muscle weight per 100 gm body weight						
	Gastroc (R) gm	Gastroc (L) gm	Soleus (R) mg	Soleus (L) mg	Plantaris (R) mg	Plantaris (L) mg
Rat 1	0.603	0.609	41.7	39.1	115.1	115.6
Rat 2	0.544	0.575	37.4	33.0	110.6	102.3
Rat 3	0.569	0.557	37.8	38.0	103.8	100.1

^aIncluding adrenals, thymus, heart, testes, kidneys, liver, and gastrocnemius, soleus, and plantaris muscles (upper 2 rows). Bilateral organs were weighed together, whereas bilateral muscles were weighed separately. Wet weights of dissected tissues normalized to 100 gm of body weight at euthanasia are in lower 2 rows. Body weights at euthanasia were 384.1 gm for the nonimplanted-operated animal, 408.8 gm for the control animal, and 386.5 gm for the implanted animal (less the weight of the implant).

Discussion and Recommendations

General

The implantation of a 3-cc transmitter and three 5-cc dummy implants into a 240-gm rat had no obvious effects on the animal's health or behavior. Measures of weight gain, food and water consumption, and postmortem organ weights showed no clear differences from the control and nonimplanted-operated animals. Measures of animal temperature and activity rhythms were similar to those reported in animals implanted with devices 3–5 cc in volume (6–10 gm).

In the implanted animal, the 5-cc implant placed between the shoulder blades eventually slipped down along the left side of the animal. However, it did not appear to cause any behavioral or health problems for the rat. This implant was probably displaced from its initial position by the action of the shoulder blades during locomotion and head movements associated with feeding and grooming behaviors. Although the implant could have been secured in its initial position with sutures this would probably have had a negative impact on behavior. This location is not recommended for subcutaneous implants in future studies. In contrast, the two units implanted on either side of the spinal column, caudal to the first implant, were fairly stable, and these sites appear to be good for subcutaneous implantation.

In future studies, it is recommended that the subcutaneous implants consist of two 7.5-cc units to achieve a 15-cc condition. The implants should be held together, long axes in parallel, with dacron-reinforced silastic sheets (obtained through DSI) fixed to the implants with Dow Corning's Adhesive A. As in this pilot study, the two implants should be aligned with, and located on either side of, the spinal column and the silastic sheet laid over the spine in a saddle-like configuration. The silastic should be sutured to the underlying musculature for long-term stability.

The anesthetic cocktail specified in table 1 worked well and is suggested for use in future surgeries. Telazol is not recommended.

Physiological Data

Food and water consumption were within expected ranges, 20–30 gm/day and 25–35 ml/day, respectively, for each animal throughout most of the test period. Interestingly, the operated animal appeared to consume less water than the control animal, a difference that increased postsurgery. The water bottle for the control animal was tested and no leaks were found. Given the

elevated water consumption of the control animal both before and after surgery, it may be that this animal consumed (or wasted) an unusually large amount of water. Clearly a larger statistical sample is needed.

Animal growth rates were within expected ranges. Brief periods of negative growth rates following surgery have been observed previously (Halberg, et al., 1971). The longer period of negative growth rate following surgery for the nonimplanted-operated animal may have been the result of the large dose of anesthetic received by this animal. It did appear that the implanted animal increased in weight a little slower than the other two toward the end of the test period, but the effect was small and requires a larger sample for statistical evaluation.

The temperature signal of 37°–37.5°C, the 24-hour period, and ~0.5°C magnitude of the diurnal temperature cycle are similar to the values reported by others for animals under normal conditions (e.g., Ishihama et al., 1989).

The Dataquest software quantified signal strength changes produced by reorientations of the implanted transmitter relative to the receiving antenna. If the signal strength changed beyond a predetermined threshold, a register was incremented. These measures were used to estimate gross animal activity. Diurnal circadian activity rhythms were observable, and contained measurable frequency and phase information. In studies in which more animals are implanted, this information could be compared across animals. However, the magnitudes of the rhythms will be more difficult to compare because monitoring is done with different receivers. Notably, in the present study, there appears to be a slight difference in the magnitudes of activity values collected with the two tested receivers. For the data shown in figure 6(a), the activity varied between an average daytime value of 9 and an average nighttime value of 25 counts per 5-minute interval. The activity data collected with a different receiver and shown in figures 6(b) and 6(c) had average values varying between 5 (day) and 17 (night) counts per 5-minute interval. These different measures, from the same animal, suggest a difference in the threshold setting on each receiver (note this can be manually set). In future studies where animal activity is derived from rf signal strength changes and where it is desired to compare activity magnitudes between animals, the thresholds on all receivers must be closely matched.

Animals were not always left undisturbed. Food and water consumption were monitored and animals were weighed. During this time the temperature/activity data acquisition continued. If data acquisition occurred when the animals were out of their cages this resulted in a dropout. Also, following handling, animal activity and

body temperature were often elevated. Animal room maintenance people periodically cleaned the room, which could also lead to modified animal activity and body temperature. It is recommended that, in future investigations of this sort, these activities be limited and take place in the evenings if possible, and all in/out times and activities in the animal room be recorded and occur at the same time each day. If possible, the data acquisition system should be located outside the animal room to reduce disruption to the animals.

On the whole, there were no large (>26%) differences between animals for the weights of any of the organs obtained (thymus, heart, liver, and left and right adrenals, testes, and kidneys). Specifically, there was no hypertrophy of the adrenal glands in the implanted animal, suggesting that this rat was not experiencing excessive stress or discomfort compared to the control and nonimplanted-operated animals. Nor were there any notable differences in the normalized wet weights of the gastrocnemius or soleus muscles between the control and implanted animal, suggesting that these weight-bearing muscles were not greatly affected by the 15% body weight increase imposed by the implants. However, a larger statistical sample is required to verify these conclusions.

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Appendix A

ACUC Protocol #92-037A

Summary

An Animal Biotelemetry System (ABS) is being designed to acquire physiological measurands from experimental rats on Space Station Freedom. The System is being designed to meet the science requirements stated in the Centrifuge Facility Level II Science Requirements Document and in the Centrifuge Facility Flight System Specifications. Requirements relevant to the ABS include:

1. Acquisition of up to 20 channels of data per rat, of which 16 shall be acquired via RF link (Note: ABS Phase A trade studies and analyses suggest a maximum of 8 channels per animal, however, the requirement for 16 has not yet been officially changed).
2. Acquisition of measurands which require sampling rates as high as 20K samples per second.
3. All data must be tagged with animal ID, and time and date of acquisition.
4. On-orbit and ground control of data acquisition parameters is required.
5. The System shall function in a micro-gravity environment, and in an artificial gravity field (0–2g) produced with centrifugation.
6. Physiological data shall be acquirable over a 90 day mission duration.

To meet these requirements, it will be necessary to design and use implanted telemetry devices which are larger than those available on the market today. The primary goal of the present study, therefore, is to determine if a larger package can be implanted in a rat without adversely affecting the animal's health and behavior. In addition to answering this engineering question, the tests outlined below will also provide information about scientific questions. It is hypothesized that the weight of a larger implant may cause changes in metabolism, thereby affecting animal body temperature regulation. The scientific goal, therefore, is to determine the effect of the implant weight on animal mean body temperature and temperature circadian rhythms.

Rationale

Engineering

Telemetry devices used for intraperitoneal (i.p.) and subcutaneous (s.c.) implantation in rats today are typically 3–6 cc in volume and weigh 6–10 gms. These devices provide 1–4 channels of multiplexed data per animal. Data sampling rates are on the order of 100–300 samples per second (sps)/channel, and transmitter on/off is manually controlled via a magnetic wand or RF switch. The ABS is presently being designed to acquire up to 8 RF channels per animal, sample at rates near 20K sps, remotely control on/off, and provide animal & data identification. To design and build the telemetry implants to these aggressive specifications, trades are required between size, cost, and performance. Preliminary analyses suggest that an implant of ~15cc (30 gms) could support, at a reasonable cost, most of these requirements. Since it is desirable to minimize costs and maximize performance, it is necessary to determine if implant sizes can be increased to ~15cc (30 gms). The designers of the ABS telemetry implants must know beforehand the volume and mass envelope to which their designs are constrained. Developing hardware before this envelope is specified risks the chance that large amounts of money will be spent on hardware that will not be compatible with the animal.

In the tests outlined below, we propose to intraperitoneally implant 250 gm rats with off-the-shelf telemetry transmitters housed in 3 cc containers weighing ~6 gms. In addition, empty 5 cc containers weighing ~10 gms each will also be implanted subcutaneously to provide total distributed implant volumes of approximately 13 cc (3 i.p. and 10 s.c.) and 18 cc (3 i.p. and 15 s.c.), weighing 26 and 36 gms, respectively. This will increase the animal's body weight 10%–15%. It is expected that the animals will be able to tolerate this size implant. The weight increase is less than that of a pregnant rat, which can be as much as 85 gms. It is approximately the percent body weight increase realized with the 4 gm telemetry implants marketed for use in mice today. Finally, implantation of small osmotic pumps of similar sizes (2 × 6.5 cc pumps) has been accomplished in rats for 2–4 weeks without noticeable effects (e.g., Fujinaga and Mazze, 1986). The goal of this portion of the study is to implant the volumes indicated above for 6 months, and to make specific, quantifiable measurements of animal health, behavior, and adaptation.

In addition, because the Freedom-based ABS will be required to operate at acceleration levels between micro-g and 2g, these tests will first be conducted with one group of rats at 1g, and then later with a new set of animals at 2g produced by centrifugation. Rats instrumented with smaller telemetry implants easily tolerate 2g centrifugation (Murakami et al., 1991). Generally, small changes in food consumption, activity, weight, and body temperature will occur during adaptation to 2g centrifugation. It is anticipated that animals instrumented with the larger packages described here will also tolerate 2g, but the actual tests need to be conducted to verify this.

Scientific

Centrifugation (1.2–2g) is known to depress body temperature in rodents and alter amplitudes, periods, and phases of circadian temperature rhythms (Oyama et al., 1971; Murakami et al., 1991; Fuller et al., 1977). The mechanism(s) for these changes are not clear. Hypotheses include increased cardiovascular load and compression of ventral structures of the brain such as the hypothalamus. Another possibility, not previously addressed, is that the temperature changes observed during centrifugation derive from an increased load on the animal musculoskeletal system. Changes in musculoskeletal load are likely to alter the metabolic requirements of the rat, and thus produce body temperature changes. To test this hypothesis, we propose to increase an animal's weight 10%–15% with an implanted load. With the first set of animals, these tests will be conducted under normal gravity to determine if the increased load causes changes in temperature from normal. Any alterations in mean body temperature and circadian temperature rhythms will be compared to those reported with centrifugation. Additional tests will be conducted later with a second set of animals at 2g generated by centrifugation, to determine if temperature changes induced by centrifugation are modified by the implant weight. If temperature changes reported in centrifuged animals are due to increased musculoskeletal loading, these changes are likely to be enhanced in centrifuged animals with heavier implants.

Animals

A total of 60 male Sprague Dawley rats weighing 225–250 grams will be used. Thirty rats will be used in 1g experiments. Five of these animals will be used in preliminary tests and as backups. The remaining 25 animals will be split into 5 treatment groups of 5 animals each. This number of animals will allow the

statistical detection of mean animal weight differences of 20 gms across treatments using a one-tailed Student's t-test at a power of ~0.8 ($\alpha \leq 0.05$). Thirty additional animals will also be used in a second set of tests to be conducted at 2g on the centrifuge. Animal sources will be determined by the Animal Care Facility veterinarian.

Rational for species selected

The rat is the currently baselined animal model to be used in life science research in the Centrifuge Facility laboratory onboard Space Station Freedom. The ABS must therefore interface effectively with the rat. The relatively small size of this species, places limitations on the size of the telemetry implant. The engineering goal of these tests is to determine the implant size limitations up to 18 cc (36 gms).

Progress

N/A

Experiment Plan

Design

Rats will be anesthetized with pentobarbital (30–40 mg/kg, i.p.), and surgeries will be conducted aseptically. Depth of anesthesia will be verified throughout the following procedure using tail-squeeze and blink reflexes. During the initial surgeries, the ACF veterinarian will be on hand to verify adequate anesthesia. Biocompatible containers will be obtained from a vendor of telemetry implants, and will be implanted intraperitoneally (i.p.) and subcutaneously (s.c.) in male Sprague Dawley rats weighing 250 gms. In treatment group 1, a 3 cc container housing a temperature transmitter will be implanted i.p.. In treatment group 2, the 3 cc telemetry unit will be implanted i.p., and two 5 cc dummy containers, back-filled to closely match the density (~2.0 gms/cc) of actual telemetry implants, will be implanted s.c. In treatment group 3, the 3 cc telemetry unit will be implanted i.p., and three 5 cc dummy containers will be implanted s.c. In both 1g and centrifugation tests, 5 rats will be used as unoperated controls, 5 will be used as sham-operated controls, and 15 (3 treatments listed above with 5 animals each) as experimental animals. Thus, for both 1g and 2g tests, treatment groups will be as shown below.

Group 1	Group 2	Group 3	Group 4	Group 5
3cc Temp sensor (i.p.) (n=5)	3cc temp and 2 5cc dummy (n=5)	3cc temp and 3 5cc dummy (n=5)	Sham operated controls (n=5)	Unoperated controls (n=5)

Surgery

For intraperitoneal implants, a half inch incision will be made in the skin over the peritoneal cavity. The skin will be gently retracted and the underlying muscle and parietal peritoneum will be incised. A 3 cc container with a temperature telemeter will then be placed in the peritoneal cavity. The peritoneal membrane will be sutured closed (4-0, vicryl) and the skin incision closed with an animal skin clip. For subcutaneous implants, a half inch incision will be made along the dorsal midline ~1 inch behind the shoulder blades. The skin will be gently teased away from the underlying tissue, until two small pockets (~5 cm × ~1 cm) are produced; one on either side of the spinal column. A 5 cc container will be slipped into each pocket, and the skin incision closed with an animal skin clip. In the case of 3 subcutaneous 5 cc containers, the third dummy container will be slipped into a slightly enlarged pocket along side one of the first two containers.

Test Procedures and Measurements

Following surgeries, animals will immediately be evaluated based on recovery rates and visual observation. They will be individually housed, given free access to food and water, and, in order to measure endogenous temperature rhythms, placed on a continuous light schedule (L:L 30 lux). Animal temperature and activity will be continuously monitored at 10 minute intervals. Food and water consumption and animal weight will be monitored daily for the first two weeks post-surgery, and twice weekly thereafter. During the first month, 0.3–0.5 cc of blood will be drawn from the tail vein every 2 weeks to test renal and liver function. After the first month, blood samples will be taken once monthly. Blood samples will be analyzed for creatine, Blood Urea/Nitrogen (BUN), liver enzymes Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT), and for corticosteroid. Twenty-one weeks post-implantation, animals will be euthanized and a necropsy performed to determine the degree of tissue necrosis, immune response, toxic response, neoplastic response,

connective tissue adhesion, local edema, etc. in the implant area. The adrenal gland and thymus will also be weighed to check for adrenal hypertrophy and thymus atrophy, respectively.

In centrifugation tests, the same procedures as outlined in the preceding paragraph will be followed. Rats will be placed on the centrifuge 4 weeks after surgery. In contrast to 1g tests, total test duration on the centrifuge will only be one month. Following centrifugation, animals will be euthanized and a necropsies performed as outlined above.

Animals with 13 cc and 18 cc implants may exhibit visible behavioral changes, altered weight gains or activity levels, reduced food or water consumption, or changes in kidney or liver function relative to control animals. Any of these conditions will be discussed and evaluated with the ACF veterinarian and, if deemed necessary, the animal will be euthanized for necropsy. Implants in animals exhibiting no observable changes, or small, non stressful changes (verified by the ACF veterinarian), would be considered acceptable. However, necropsies would be performed at the end of the tests in order evaluate the implant and local tissue.

Test Schedule

The schedule of surgeries and experimental procedures will be as shown on the attached sheet.

Pain, Discomfort, and Distress

Every effort will be made to reduce pain, discomfort, and distress to the experimental animals. During surgical implantation of transmitters, animals will be fully anesthetized. Following surgery, post-operative analgesics and antibiotics will be given by the ACF veterinarian if indicated. Because activity and temperature are obtained via wireless telemetry, no pain will be associated with obtaining these measurands. Stress and discomfort will be minimized during animal weighing, cage changes, etc. Repeated blood samples

(0.3–0.5 cc) will be drawn from the tail of unanesthetized animals using techniques which minimize pain and stress for the animal (Omaye, et al. 1987). If an animal appears clearly distressed or uncomfortable (as determined by the ACF veterinarian), testing will be terminated and the animal euthanized for necropsy.

Hazards

Hazardous substances will not be used in these tests.

Experience and Qualifications

During his doctoral work (July, 1989), the PI gained a great deal of experience with neurophysiological recording techniques in rodents. Over the past 2 years, he has conducted surgical procedures and chronic neurophysiology experiments on squirrel monkeys as described in ACUC protocol #92-002. The surgeries required in the present tests are comparatively simple, and will be done initially under the supervision of the ACF veterinarian.

Disposition of Animals

At the conclusion of all experimental testing, animals will be euthanized by decapitation. Blood samples will be collected and tissues will be prepared as necessary for necropsy.

Statement of Compliance

ACF personnel will be consulted regarding any problems that may arise with experimental subjects. The care and use of animals used in this study will comply with the ARC Animal Users Guide, AHB 7180-1.

Responsible Scientist/Engineer

The Gravitational Biology Facility Project Scientist, Charlie Wade, will be the responsible NASA employee for these tests.

Responsible Veterinarian

The ACF veterinarian will be the responsible veterinarian.

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13. ABSTRACT (Maximum 200 words) This paper reports the results of a pilot study in which a 240-gram rat was implanted for 41 days with biotelemetry devices weighing a total of 36 gm (18 cc). The implanted animal showed no differences in weight gain, food and water consumption, and postnecropsy organ weights when compared to both an unoperated control animal and an animal that underwent surgery but did not receive an implant. The implanted animal also had temperature and activity rhythms similar to those reported using much smaller implants. Thus, this pilot study showed that a 240-gm rat could be implanted with biotelemetry devices weighing nearly 15% of body weight without significant changes in health or behavior. A larger study involving more animals and similar implant sizes is recommended.				
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